THE IDENTIFICATION OF FIVE *SEEDLINGS*

HYPER-RESPONSIVE TO LIGHT (*SHL*) AND CHARACTERIZATION OF *SHL7*

A Dissertation

by

DANIEL SCOTT GRUM

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2008

Major Subject: Genetics

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Approved by:

Chair of Committee,	Alan E. Pepper
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ABSTRACT

The Identification of Five *Seedlings Hyper-responsive to Light* (*SHL*) and Characterization of *SHL7*. (August 2008)

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Chair of Advisory Committee: Dr. Alan Pepper

Light is one of the major environmental factors that controls plant development, through a process known as photomorphogenesis. Plants perceive light via photoreceptors, and the information used to direct a myriad of developmental responses. Analysis of mutants defective in photomorphogenic responses elucidates the complex interactions between light and plants. Previous genetic screens have yielded a class of mutants which exhibit exaggerated responses to ambient light, designated *shl* (*seedling hyper-responsive to light*). The following work encompasses the identification of five new *shl* mutants, a detailed examination of one of these mutants (*shl7*), and of the *SHL7* gene. The mutants were isolated in a low-white light screen of seedlings derived from T-DNA mutagenesis. Each of the mutants exhibits a heritable hyper-responsive phenotype in low-white light, but displays minimal effects in darkness. For each, a putative site of T-DNA insertion has been located. In addition to a low-white light phenotype, the s*hl7* mutant exhibits a mild hyper-responsive phenotype to 670 nm red and 735 nm far-red light, but significant hyper-responses to 420 nm blue light. *SHL7* encodes a small, unique, and previously undescribed protein annotated as At4g04925. GFP protein fusion analysis indicates that the protein is localized to mitochondria.

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CHAPTER I

INTRODUCTION

To cope with a sessile lifestyle, plants have highly plastic morphologies that are governed by responses to the specifics of the environment, including gravity, temperature, and light. Light, the primary source of energy used by plants, has driven the evolution of an array of sensory systems capable of acute perception and analysis of its properties, including irradiance, spectral quality, direction, and periodicity (Fankhauser and Chory, 1997). The developmentally plastic nature of plant morphology is instrumental in aiding the efficiency of light capture. Plants adjust their growth and development to optimize their harvest of available radiant energy, a process termed photomorphogenesis.

After germination, the success of a seedling is contingent upon its capacity to assess its environment and appropriately choose between a skotomorphogenic or a photomorphogenic developmental pattern (Mohr *et al.*, 1983). In darkness, a seedling will develop according to the skotomorphogenic pathway wherein the seed's energy is allocated strictly towards locating light (Steindler *et al.*, 1999). Skotomorphogenesis in *Arabidopsis* is characterized by an etiolated growth pattern. Visible phenotypic characteristics include a closed apical hook, closed and unexpanded cotyledons, and rapid elongation of the hypocotyl.

Exposure to sufficient light will induce a photomorphogenic pathway. UV-A/blue, red, and far-red light are effective in triggering the change. In the seedling,

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This dissertation follows the style of *The Plant Journal.*

energy allocation is directed towards establishing the plant as a photoautotrophic organism through de-etiolation (Quail, 2002). De-etiolation is marked by the straightening of the apical hook, unfolding and expansion of the cotyledons, the induction of chloroplast development and increased pigmentation including the synthesis of chlorophyll and anthocyanins (Lin, 2002), the suppression of hypocotyl elongation, and a massive reorganization of the transcriptional program (Liscum *et al.*., 2003).

Direct responses to light are mediated by photoreceptors. In higher plants, several classes of photoreceptors have been identified, each of which perceives light of particular wavelengths within the photoactive spectrum. These classes include cryptochromes, phototropins, and phytochromes. Cryptochromes (CRY1 and CRY2) and phototropins (PHOT1 and PHOT2) monitor the blue/ultraviolet region of the spectrum, while phytochromes (PHYA-PHYE) typically monitor the red/far-red region (Quail, 2002).

Cryptochromes and phytochromes coordinately control seedling establishment, entrainment of the circadian clock, and the transition from vegetative to reproductive growth (Chen *et al.*, 2004). The cryptochromes are very influential photoreceptors during de-etiolation, with CRY1 and CRY2 playing partially redundant roles. CRY1 is the primary photoreceptor under high-blue fluence rates and CRY2 plays a more important role in low-blue fluence rates. CRY1 is stable in high-intensity blue light, whereas CRY2 is rapidly downregulated by blue light in a light-intensity-dependent manner (Lin *et al.*, 1998). Cryptochromes are also important for photoperiod-dependent flowering induction and in resetting the circadian oscillator (Cashmore *et al.*, 1999). These physiological processes are controlled by interconnected networks of both cryptochromes and phytochromes. While the cryptochromes and phytochromes share

involvement in seedling establishment and floral induction, the phytochromes are considered to be solely responsible for the control of seed germination and shade avoidance responses (Neff *et al.*, 2000). Phototropins control phototropism, a process primarily involving the bending of stems towards light, and negative phototropism, demonstrated by roots growing away from light. Phototropins are also involved in the relocation of chloroplasts within cells to maximize photosynthesis (Sakai *et al.*, 2001).

Light perception in plants has been studied for over a century, with the discovery of phototropism as a blue light-mediated response in the 1800's (Darwin, 1880). The majority of our knowledge, however, has been accumulated in the past few decades, with much resulting from breakthroughs in the relatively new field of genetics and the use of modern molecular methods. *Arabidopsis thaliana* has been the model plant system for studies in environmental interactions in plant development, with light perception being the subject of intensive genetic analysis. An abundance of information concerning photoreceptors has been gained from studies of *Arabidopsis* mutants with altered functionality in one or more of the photoreceptor genes, allowing researchers to identify which photoreceptors control particular aspects of light-regulated development (Sullivan and Deng, 2003).

In addition to the identification of the primary photoreceptors, the signaling events downstream of these photoreceptors account for much of the focus of current research into light-regulated plant development. The majority of our understanding of downstream signaling events comes from the examination of de-etiolation in *Arabidopsis* seedlings (Quail, 2002). The developmental changes seen during de-etiolation are the result of a change in expression of approximately 30% of the genes in the *Arabidopsis*

genome (Ma *et al*., 2001). Many components of the signaling pathways downstream of the photoreceptors have been discovered through the identification of mutants defective in various aspects of de-etiolation.

De-etiolation involves a massive reorganization of the transcriptome. This large-scale genomic and morphological transition offers a variety of avenues for experimental gene discovery using sophisticated techniques. However, many of the most important components of light regulation have been identified through relatively simple mutant screens for deviations in hypocotyl lengths under various light regimes and conditions. Screens for plants with long hypocotyls in various light conditions, and for short hypocotyls in dark have been particularly effective. The *hy* (*elongated hypocotyl*) mutants *hy1* and *hy2* are long hypocotyl mutants which have demonstrated HY1 and HY2 involvement in phytochrome chromophore biosynthesis (Parks *et al*.,1991). The mutant *hy3* was found to be deficient in phytochrome B (Somers *et al*., 1999) and has been renamed *phyB*. The mutant *hy4* was originally found in an abscissic acid-insensitive screen (Korneef, 1984). The HY4 protein was isolated and found to resemble a bacterial photolyase (Ahmad and Cashmore, 1993) and is now known to be the cryptochrome CRY1. HY5 is a b-zip transcriptional activator which is destabilized in the dark, where it is degraded by the COP1- mediated proteasome. In light, HY5 is required for the expression of a number of light- responsive genes (Osterlund *et al*., 2000). HYH is a HY5 homolog and is also a b-zip protein degraded by the COP1- mediated proteasome. The HYH protein is dependent on the presence of HY5, and can possibly form a heterodimer with it. HYH is predominantly involved in blue-light regulation of development and gene expression (Holm *et al*., 2002). Short hypocotyl mutants include

the *cop* (*constitutive photomorphogenisis*), *det* (*de-etiolated*), and *fus* (*fusca*) mutants. The majority of COP/DET/FUS proteins have turned out to be members of the COP9 signalsome, or CSN, with the notable exceptions of COP1 and DET1.

Among the most influential non-photoreceptor genes identified as important for de-etiolation are the *COP*/*DET*/*FUS* genes, which are necessary for the repression of photomorphogenesis in darkness (Wang and Deng, 2002), and *HY5* and *HYH*, which act as positive regulators of photomorphogenesis. COP1 functions as a light-inactivatible repressor of photomorphogenesis, and shows nuclear enrichment in darkness. It has been shown to interact with transcription factors such as HY5 and HYH. Defects in the *COP*/*DET*/*FUS* genes result in constitutive photomorphogenesis and the de-etiolation of *Arabidopsis* in complete darkness, whereas defects in *HY5* result in a failure to properly de-etiolate in high-light conditions.

The removal of many of these fundamental components of light signaling processes result in constitutive photomorphogenic phenotypes, or complete insensitivities to particular spectrums of light. There are, however, an extensive number of genes involved in light perception. More refined screens are used to discover several more specific players in signal transduction. Screening mutants in various intensities of red, far-red, and blue light has led to the discovery of many mutants with defective responses to light. These mutants often have wild-type phenotypes in darkness where there is no light to respond defectively to. Mutants have been identified which respond in a hypersensitive manner to lights of varied qualities, providing information about the specific roles of the cryptochromes and phytochromes in plant development- as well as where their functions overlap. These defective responses of these mutants to particular

lights include premature deetiolation as compared to their wild-type counterparts-resulting in a physically shorter hypocotyl. Among these are *shb1 (short hypocotyl under blue1*), which exhibits a short hypocotyl when grown under blue light. Overexpression of the wild-type *SHB1* gene causes long hypocotyl phenotypes in red, far-red, and blue light (Kang and Ni, 2006). The mutant *hrb1* (*hypersensitive to red and blue1*) has a hypersensitive hypocotyl growth response to red and blue light (Kang et al., 2005). The hypocotyl response of the mutant approaches saturation at much lower intensities of light than that of the wild type. The *psi2* mutant is hypersensitive to red and far-red light (Genoud et al., 1998) with defective deetiolation responses in both conditions. One particular screen involving treatments of alternating red and far-red light pulses isolated a series of mutants which were called *eid* (*empfindlicher im dunkelroten Licht*, which means hypersensitive in far-red light). Among this group, *eid6* turned out to be a novel recessive allele of the *COP1* gene. These $cop1^{eid6}$ mutant seedlings exhibited extreme hypersensitivity towards all tested light qualities, but in contrast to known *cop1* alleles, no constitutive photomorphogenic phenotype was detectable in darkness (Dieterle et al., 2003).

In a previous study (Pepper et al., 2001), a new screen for EMS-generated mutants with phenotypic effects in low light - a threshold condition in which photomorphogenetic signaling pathways are only partially active - identified mutants with exaggerated developmental responses. These mutants differ from the *cop*/*det*/*fus* class as they do not constitutively de-etiolate in darkness. They are designated *shl* for *seedlings hyper-responsive to light* (Pepper *et al*., 2001). Mutants in this category show enhanced responsiveness to low-white, red, far-red, and blue light, but minimal

phenotypic effect in darkness. The *SHL* genes act genetically as light-dependent negative regulators of photomorphogenesis and do not appear to be weak alleles of *cop det* or *fus* loci. Fifteen *shl* mutants were identified and assigned to complementation groups by F_1 complementation analysis, with four mutant lines falling into mono-allelic complementation groups, indicating the screen was not exhaustive (Pepper *et al*., 2001).

Early experiments involving large- scale mutagenesis were accomplished by randomly damaging the DNA of multitudes of organisms using radiation or mutagenic chemicals. While an effective means of generating massive quantities of mutant individuals, the mutations are confined to deletions or individual nucleotide changes. Physiological alterations wrought by these types of genetic changes typically come in the form of non-sense or mis-sense mutations that can destroy a protein's functionality or prevent its synthesis altogether. The complete loss of the function of a gene is referred to as a "knock out" mutation. Knock out mutations have provided a wealth of information about genes for organisms from every kingdom. There are limitations, however to the extent of information obtainable from knock outs. Genes which are necessary for the most basic tasks of living cannot be studied using knockout mutations, at least beyond the observation that the mutation is lethal.

In *Arabidopsis*, efficient means of introducing exotic DNA sequence into the genome, particularly using *Agrobacterium*, has led to new possibilities for mutagenesis screening. T-DNA insertions are used as the source of genetic changes which can lead to mutation types other than knock-outs. A T-DNA containing enhancers, or a promoter sequence can turn on, or over-express nearby genes, a technique called activation tagging. Activation tagging allows for the identification of genes based on their

overexpression phenotypes which allows for the tagging of genes which are lethal when knocked out (Weigel *et al*., 2000).

The following work encompasses the identification of five new *shl* mutants, and a detailed examination of one of these mutants (*shl7*), and the *SHL7* gene. The mutants were isolated in a low-white light screen of seedlings derived from a T-DNA mutagenesis. Each of the mutants exhibits a heritable hyper-responsive phenotype in low-white light, displays minimal effects in darkness, and has had a specific site of insertion for the T-DNA located. In addition to a low-white light phenotype, the s*hl7* mutant exhibits a mild hyper-responsive phenotype to varied fluences of red and far-red light, but exhibits significant responses to blue light. *SHL7* encodes a small unique protein annotated as At4g04925. At4g04925 does not appear to be related to any known genes in any Kingdom. The hypothetical protein shares no homologous structures with other characterized proteins using threading for protein analysis. The protein contains a mitochondrial localization peptide sequence, and sub-cellular localization to the mitochondria was confirmed using a GFP/Protein fusion. No detectable localization was discovered in any other organelles within the cell. The mitochondrion is a very influential organelle in plant growth and development, beyond being the "powerhouse" of the cell. Elucidating the roll of this interesting, small, and very unique protein within these organelles has much potential to yield equally interesting and unique insights.

CHAPTER II

IDENTIFICATION OF T-DNA TAGGED *shl* MUTANTS

INTRODUCTION

The environment has great influence on the developmental morphology of higher plants. Of the environmental factors which shape this growth and development, light is unique in that it provides the energy used for autotrophic growth in the plant kingdom. Thus, special consideration is given to light as an environmental regulator. Light drives the process of photosynthesis, but also contributes to the governing of a plants growth habit to optimize light capture, a process termed photomorphogenesis. Specifics about the ambient light conditions are gathered by light-absorbing photoreceptors. Photomorphogenesis affects nearly every fundamental aspect of a plants existence, so understanding precisely how it works is a daunting task. Changes resulting from photomorphogenesis are not limited to visible morphological characteristics, but also occur at the genetic level and are detectable as changes in the gene expression of components in signaling pathways downstream of the photoreceptors.

This study focuses on the genetic components of photomorphogenesis, and is carried out using seedlings undergoing de-etiolation. De-etiolation is perhaps the first photomorphogenic response which occurs in a seedling. In *Arabidopsis thaliana*, it involves the reorganization of 30% of the transcriptome (Ma et al., 2001). De-etiolation is a conspicuous event, which provides an excellent opportunity for the study of light

regulation. The majority of what is known about downstream signaling events has come from the study of *Arabidopsis* mutants with defects in de-etiolation response.

With the goal of identifying new genes involved in light regulation of plant development, a mutational approach was taken to discover new genes with functions necessary for the proper response to controlled light conditions. To identify new genes specific to the photomorphogenic pathways, a large scale screening of T-DNA insertional mutants was carried out under low-white light conditions. This would constitute a threshold condition of the photoactive spectrum wherein the photomorphogenic response pathways of any photoreceptors would be only partially active. Previous studies have identified mutants in this light regime, which have been named *seedling hyper-responsive to light*, or *shl* mutants. This screening of T-DNA insertional mutant lines successfully identified new mutants which appear to be deficient in light perception and/or response.

RESULTS

Mutant screen

To identify new members of the seedling hyper-responsive to light group, seed pools from 84,000 individual T_1 T-DNA lines donated by Dr. Rick Amasino and Dr. Scott Michaels (University of Wisconsin - Madison) were screened. Several *Arabidopsis* light-responsive mutant seedlings were identified during a low-fluence white-light screen, that had de-etiolated morphologies with enhanced inhibition of hypocotyl elongation after 7 days of growth. This screening of T-DNA mutants identified multiple new *shl*-type

mutant lines. The five lines displaying the most robust phenotypes were selected for further analysis.

These lines were designated 5C, 9B, 12B, 42A, and 57F. Each of these mutant lines was propagated through multiple generations to confirm that the respective phenotype was heritable. Each of the lines retained a *shl* phenotype in consecutive generations. To verify the presence of T-DNA in each line, individuals were selected for resistance to the herbicide Basta. The *shl* mutants displayed shorter hypocotyl lengths than ecotype Columbia when grown in low-white light conditions (12 μmol m^{-2} s^{-1}), and were counter-screened in darkness where they showed negligible hypocotyl length deviations from Columbia. These findings were consistent with the *shl* description, separating them from the *cop*/*det*/*fus* class.

To further test these lines, backcrosses of homozygous lines to the wild-type Col-0 were performed. Segregation in the F_2 populations of the Basta resistance, conferred by the T-DNA insertions, and of the *shl* phenotype were examined. Segregation ratios of the herbicide resistance confirmed a single genetic locus insertion of functioning T-DNA in each line. Segregation ratios of the mutant *shl* phenotype met the statistical expectations of a functionally recessive trait in four of the five mutants (Table 2.1).

Table 2.1 F_2 segregation ratios on basta, and mutant phenotype segregation.

Line	Alive:Dead	Chi dist	WT:*shl*	Chi dist
5C	60:20	χ^2=0.0	47:17	χ^2=0.08
9B	54:18	χ^2=0.0	37:14	χ^2=0.16
12B	33:14	χ^2=0.57	31:27	χ^2=14.37
42A	59:21	χ^2=0.07	45:16	χ^2=0.05
57F	33:6	χ^2=1.97	123:32	χ^2=1.57

Chi distributions for Basta resistance represent an expected 3:1 resistant to susceptible

Chi distribution for *shl* phenotype represent as expected 3:1 wild-type to *shl*

The F_2 segregation of the 5C line for Basta resistance was 60:20 with a χ^2=0.0 for an expected single locus insert, and segregation of the *shl* phenotype, wild-type to mutant was 47:17 with a χ^2=0.08 for an expected recessive allele. The segregation of the 9B line for Basta resistance was 54:18 with a χ^2=0.0 for an expected single locus insert, and a 37:14 wild-type to *shl* phenotype segregation with a χ^2=0.16 for an expected recessive allele. The segregation of the 12B line Basta resistance was 33:14 with a χ^2=0.57 for an expected single insert, but the segregation of *shl* phenotype did not match the statistical expectations of either a recessive or dominant allele. The F_2 progeny displayed a range of phenotypes which were not found in a 1:3 ratio expected of a recessive or a 3:1 ratio expected of a dominant, or a 1:2:1 ratio expected of distinct semi- dominant effect. The

ratio of wild-type to mutant was 31:27 with a χ^2=14.37, indicating something other than a simple, single gene knock-out. The segregation for Basta resistance ratios for the 42A line was 59:21 with a χ^2=0.07 for an expected single locus of insert, and 45:16 for wild-type to *shl* with a χ^2=0.05 for a recessive allele. The segregation ratios for Basta resistance for the 57F line were 33:6 with a χ^2=1.97 for a single locus of insert, and 123:32 for wild-type to *shl* with a χ^2=1.57 for a recessive allele.

T-DNA insert locations

TAIL-PCR and plasmid rescue techniques identified *Arabidopsis* genomic sequences flanking the T-DNA insertion sites in each of the five mutant lines. Basta segregation ratios of F_2 backcrosses indicated a single function locus T-DNA insertion in each line. Recovered sequences were consistent with a single locus of insertion. The sequences were blasted against the TAIR databases and the chromosomal locations were determined (Figure 2.1). The 9B, and 12B T-DNA insertions were located on the short arm and long arm of chromosome 4 respectively, and the 5C, 42A, and 57F T-DNA insertions were located on chromosome 5, with 42A and 57F on the short arm and 5C on the long arm.

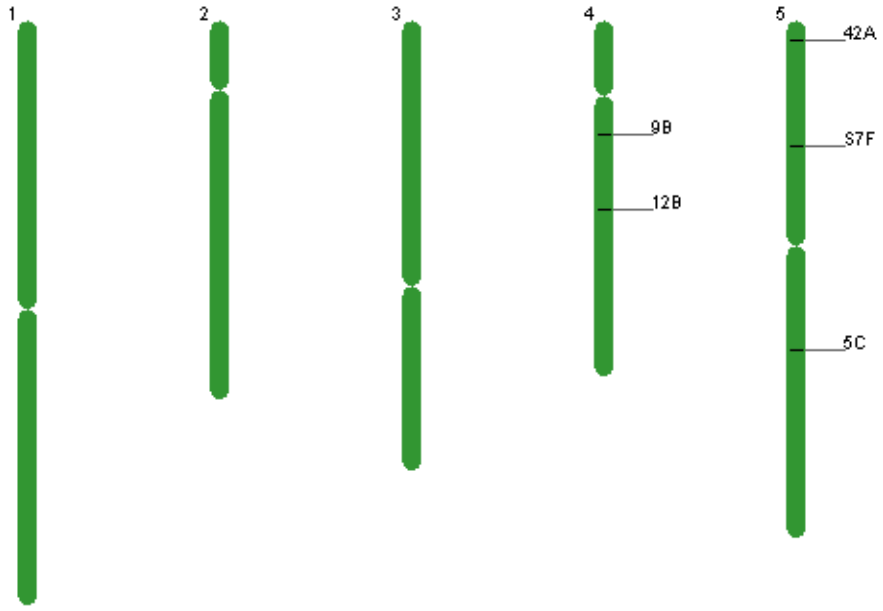


Figure 2.1 Putative chromosomal locations of T-DNA insertions.

(a)

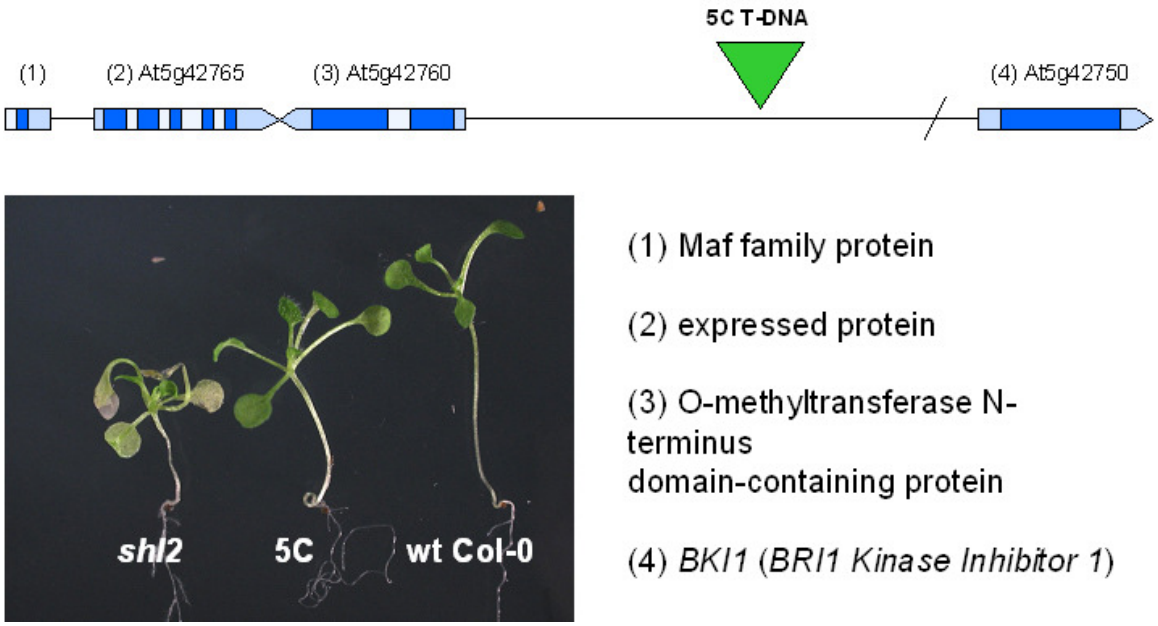
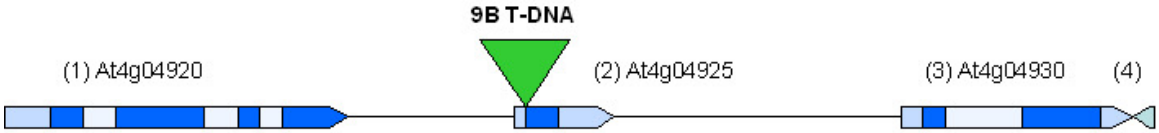


Figure 2.2 Morphologies of selected mutant lines. Seedlings were grown for 7 days on Morishige and Skoog/phytagar/2% sucrose media. low-white light 12 µmol $m^{-2}s^{-1}$. Locations of T-DNA inserts are shown to scale depicting 10Kb of DNA.

(a) 5C in yellow filtered light, the horizontal line indicates an additional 623bp
(b) 9B in low-white light
(c) 12B in low-white light
(d) 42A in low-white light
(e) 57F segregating F_2 *shl* and wild-type in low-white light

(b)



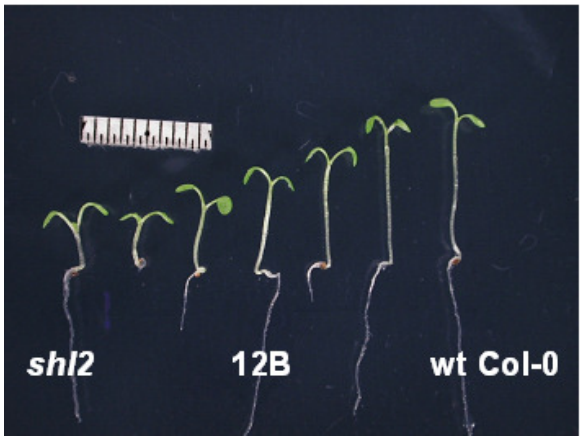
(1) expressed protein

(2) expressed protein

(3) Transmembrane fatty acid desaturase

(4) transducin family WD-40 repeat family G protein

(c)

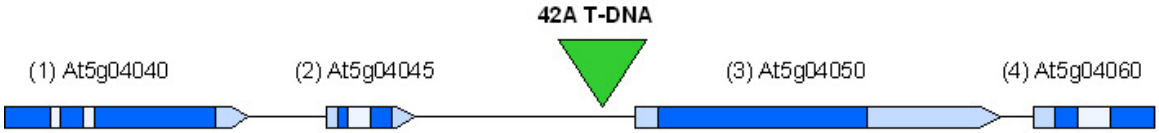


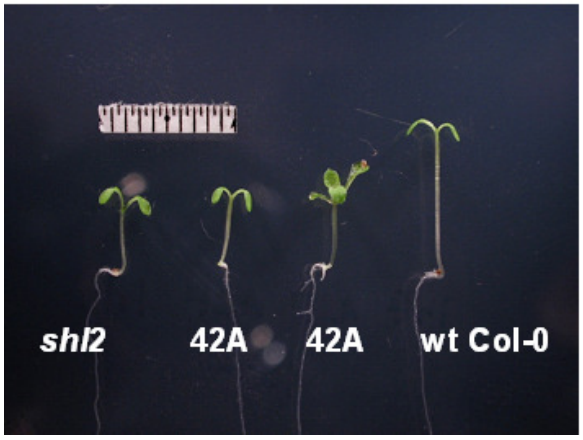
(1) similar to protein kinase

(2) similar to senescence-associated protein-related

Figure 2.2 continued

(d)





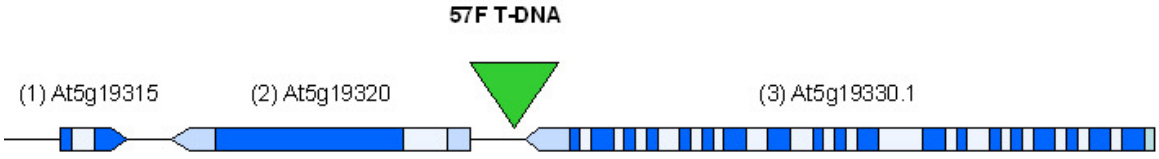
(1) SUGAR DEPENDENT1 SDP1

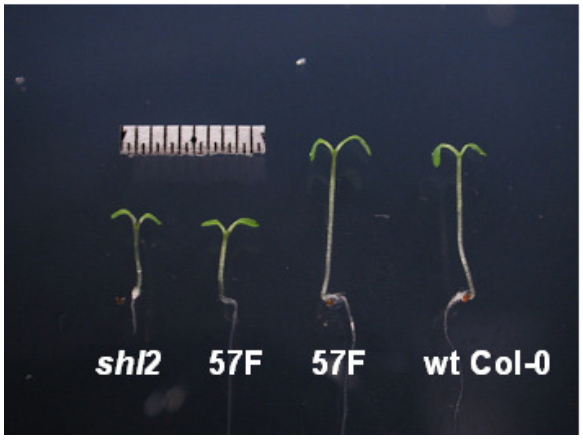
(2) expressed protein

(3) maturase-related

(4) similar to early-responsive to dehydration stress ERD3 protein

(e)





(1) defensin-like (DEFL) family protein

(2) RAN GTPase activating protein 2 RANGAP2

(3) arm repeat interacting with ABF2 (ARIA)

Figure 2.2 continued

DISCUSSION

Five new *shl*-type mutants were obtained from this T-DNA mutagenesis screen for which the T-DNA insertion sites have been identified. None of these mutations are redundant with any of the located mutations from the previously defined *shl* mutants originating from the EMS-generated mutant lines, nor are any redundant among the T-DNA derived lines. This would indicate that the screening for *hyper-responsive to light* class mutants in this light regime is not exhausted. It can also be noted that none of the previously described constitutively photomorphogenic class of mutants, or weak alleles thereof were obtained. This particular screening yielded many potential *shl*-type candidates, of which this subset of five mutants were selected for futher analysis. Further study of these remaining candidates will likely reveal additional unique members of this group of mutants.

Each of the five T-DNA mutants studied here had a single apparent site of insertion for functional T-DNAs, which tracked with the *shl* phenotypes through multiple generations, implicating that T-DNA as the cause of phenotype. Further work is required to substantiate these insertions beyond doubt that they are lone mutations, but for the purposes of this discussion, they will be treated as so. Of the five insertion sites, only one, 9B, is clearly in an annotated gene. Two out of the five insertion sites, 5C and 12B, are not within any annotated gene, nor have any corresponding ESTs or cDNAs been identified to support the hypothesis of a knock-out. The 5B mutant segregation follows a recessive pattern typical of a knock-out, so it is possible that it is disrupting some sort of functional RNA. Another possibility is that it may be activating the expression of a

distant gene in a more convoluted manner. Preliminary attempts to identify possible miRNAs in the 5C insertion region based off of known honeybee miRNA structures were inconclusive(). The assumption being that the structure of miRNAs may be conserved between these species. The 12B mutant displays a possible semi- dominant phenotype consistent with what might be expected of an activation tag. The remaining two mutant lines, 42A and 57F, have insertion sites that plausibly disrupt the promoter elements of adjacent genes, and both lines segregate as recessive alleles. These data tend to support a single gene knock-out hypothesis for the effect of the mutations.

Possible mechanisms for effecting shl-like phenotypes and future work

The immediate priority of future work concerning these mutants is to conclusively demonstrate that the phenotypes of these mutants are linked to their respective T-DNA insertions. Each line has been observed through multiple generations from several progenitors, and each retain a basta resistance. This is suggestive that the basta resistance and the *shl* phenotype are linked, but does not constitute concrete evidence. Southern blots to identify the possible presence of additional incomplete T-DNA transfers not detectable via basta selection will need to be performed. However, as the particular insertion sites containing full T-DNAs are confirmed, the discussion of the following mutant lines may become quite applicable, pending more substantial evidence indicating the absence of additional mutations.

Line 5C

The T-DNA insert in line 5C is not in an annotated gene, but is within 1kb of the annotated gene At5g42750. At5g42750 has been designated as *BKI1* for *BRI1 Kinase Inhibitor1* (Wang *et al*., 2001). *BKI1* encodes a plasma-membrane associated phosphoprotein that interacts directly with the kinase domain of BRI1 (Brassinosterioid-Insensitive1). BKI1 interferes with the interaction between BRI1 with its signaling partner, the plasma membrane localised LRR-receptor kinase BAK1. BRI1 is a receptor for brassinosteroids and transduces steroid sinals across the plasma membrane by means of receptor kinase activity (Wang and Chory, 2006).

Brassinosteroids (BRs) are important hormones that regulate growth and development. Two classes of BR mutants have been widely studied: one class is impaired in BR biosynthesis, and the other class is predicted to be blocked in the perception of BRs or in essential components of BR signaling downstream of perception. All BR mutants are dwarfs. BR mutants deficient in BR biosynthesis can have their phenotypes rescued by the application of exogenous brassinosteroids, whereas BR-insensitive mutants will not respond to brassinosteroid feeding (Noguchi et al., 1999). *BRI1* is required for BR perception at the cell surface, so a *bri1* mutant will not respond to BR feeding.

A *bak1* knockout mutation has been shown to give rise to a weak *bri1*-like phenotype (Nam and Li, 2002). If the CaMV enhancers on the T-DNA insertion are up-regulating *BKI1*, an overexpression of this protein, which interacts directly with the kinase domain of BRI1 and interferes with the interaction between BRI1 and BAK1, could plausibly yield a weak *bri1*-like dwarf phenotype. To follow up on this hypothesis,

future projects would include quantitative PCR of the *BKI1* transcript to confirm any up-regulation of the gene, and a BR-feeding experiment on the 5C mutant. If an anomalous amount of the BKI1 protein is interfering with BRI1's interactions with BAK1, it should respond to light in a manner similar as a BR insensitive mutant.

Line 12B

The 12B mutant phenotype exhibited a range of expressivity in its phenotype. The wild-type to mutant ratio does not, at first glance, match any clear dominant or recessive pattern. The mutants, however, were considered to be any plant that was clearly "not wild-type", so the range of expressivity seen could reasonably place mutant plants into the wild-type category. A likely explanation is that the 12B phenotype is a dominant one resulting from an over-expression driven by the CaMV enhancers on the T-DNA, which was designed to be an activation tagging vector. The activation tagging effects of such insertions have been shown to potentially influence gene expression over large distances, but the nearby genes are logical starting points.

While the nearest annotated gene At4g17670, described as similar to senescence-associated protein-related, might be involved in the light hyper-responsive phenotype, At4g17660 a protein-kinase related gene seems an equally promising candidate. It is possible that as much as a third of all proteins may be modified by kinase activity (). Kinases are known to regulate some part of the majority of cellular pathways, especially those involved in signal transduction, so the hypotheses for what this protein does are relatively inexhaustible. Future experiments would include quantitative PCR of both of these genes, for a comparative expression study against the wild-type. A putative open

reading frame was also identified at the site of insertion using the Genefinder (Cold Spring Harbor Laboratory) software, but there is no corroborating EST or cDNA evidence to support the hypothesis of a gene at this locus.

Line 42A

The 42A insert is located approximately 259 bp from the 5′ end of an annotated maturase- related gene At5g04050, in what is putatively the promoter region. This proximity to At5g04050, combined with the recessive nature of the mutation makes this maturase related gene a likely candidate for this *shl* gene. Two available SALK lines, SALK 139392 and SALK 011307, which are located within 180 bp of the 42A T-DNA insertion were examined. Neither SALK line appeared able to recapitulate the *shl* phenotype (data not shown). In light of the SALK line information, At5g04050 still remains a possible candidate for further study. The priority of future work should entail quantitative PCR of the At5g04050 transcript in the 42A mutant to determine whether transcription is indeed knocked down or knocked out.

In the event At5g04050 is not knocked out, an alternative hypothesis arises from the 42A insert's location that is 2500 bp downstream of At5g04040, or *SUGAR-DEPENDENT1* (*SDP1*). Mutations in *SDP1* have been previously shown to result in retarded seedling development. Seed germination is followed by a phase of rapid growth as the seedling strives to successfully establish itself. This growth is fueled by storage reserves in the seed. Oil within the seed is the most common storage compound in seeds. The initial step of oil breakdown is catalyzed by lipase, which hydrolyzes triacylglycerol (TAG) to yield free fatty acids and glycerol. These are ultimately converted into sugars.

SDP1 is an oil body–associated TAG lipase that is responsible for catalyzing the initial step in storage oil mobilization in Arabidopsis seeds (Eastmond, 2006). A *sdp1* mutant is not significantly impaired in germination. However, the mutant *sdp1* exhibits a much slower rate of postgerminative growth than the wild type. This retarded growth is rescued by a supplemantal provision of sucrose. Once *sdp1* seedlings develop photosynthetic activity, they grow normally and are indistinguishable from the wild-type throughout the rest of their life cycle (Eastmond, 2006). *SDP1* transcript levels do not correlate positively with enzyme activity. The likely explanation for this discrepancy is that SDP1 is regulated at the posttranscriptional level (Eastmond, 2006). The 42A activation-tagging insert near the SDP1 locus could be altering the normal expression levels of this gene. Knockouts of *SDP1* resulted in retarded growth of seedlings, but over-expression of the gene has not been examined. It is possible that over-expression of SDP1 could trigger a post-transcriptional silencing response. It should be noted, however, that the media used in this particular experiment contained sucrose which could restore a wild-type phenotype to *sdp1* knockouts. Future experiments with this mutant might include quantitative PCR of the At5g04040 (SDP1) transcript to check for anomalies either above or below the norm, and a replating of the 42A mutant in low-white light and the dark on media bereft of sucrose.

Line 57F

The 57F T-DNA insert is 323 bp upstream of RanGTPase-activating protein2 (RanGAP2) which plays a role in nucleocytoplasmic transport, mitotic spindle formation, and nuclear envelope assembly through its association with Ran (Zhao et al., 2006).

Ultimately RanGap proteins are involved with the transport of proteins and RNAs between the nucleus and cytoplasm. The location of the 57F insert, in the putative promoter region of RanGap2, places it as the immediate candidate gene for further study. The clearly potent nature of the protein and the recessive segregation of the 57F phenotype suggest a knock-out of RanGap2 as the leading hypothesis for the *shl* phenotype. Quantitative PCR to confirm the down-regulation of this gene would be the initial step in future experiments.

A secondary hypothesis would involve At5g19330 which is the *ARIA* gene. The 57F T-DNA insert is 100 bp from the 3′ end of At5g19330. ARIA is an ABA signaling component that regulates seed germination, seedling growth, glucose response, and ABA and/or stress response (Kim et al., 2004). The *ARIA* gene appears to be a tempting candidate for the 57F line, but the physical location of the T-DNA near the 3′ end does not provide clear explanation for how the mutant phenotype arises. Previously studied *ARIA* over-expression lines did not exhibit significant growth phenotypes under normal conditions except slightly (approximately 1 h) delayed germination (Kim et al., 2004). Knockout mutations of ARIA share similar phenotypes to the wild-type, but hypocotyls growth in low-light conditions has not been examined. The T-DNA insert of pSKI015 has been shown in previous mutants to give rise to an mRNA spanning the LB into the plant DNA(). This could provide a reverse complement template for the *ARIA* gene and draw the attention of gene silencing mechanisms resulting in a knockout phenotype. Future work would include plating SALK line T-DNA knockout lines in low-white light conditions and quantitative PCR of the *ARIA* gene expression in the 5C line compared to wild-type.

EXPERIMENTAL PROCEDURES

Plant lines and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used in all experiments. Plant lines with T-DNA insertion mutations were obtained from Dr. Rick Amasino and Dr. Scott Michaels (University of Wisconsin - Madison). One hundred and twenty T2 seed pools were obtained from 84,000 T1 lines, which were created by an agrobacteria-mediated mutagenesis utilizing the pSKI015 (Weigel *et al*., 2000) activation-tagging vector. Plants were grown in soil (Metromix 300) or on MS/phytagar/2% sucrose media in 25 mm x 100 mm polystyrene Petri dishes. Seeds were surface sterilized (Chory *et al*., 1989), resuspended in 0.1% phytagar, and cold-treated for 48 hours prior to plating. Seeds were evenly dispersed onto solid agar media in a 7mm grid pattern, and then subjected to 4 hours of white light (100 μmol m^{-2} s^{-1}) prior to placement into specified light conditions. For white and low-white light conditions, plants were placed in a temperature- and humidity-controlled growth room under GE Cool White and Sylvania Gro Lux lights at 22±1°C. Neutral density filters were used to adjust fluence rates for low-white light. Fluence rates of white light were measured with a quantum photometer (model LI-189, LI-COR, Lincoln, NE).

SALK lines

Information regarding the SALK lines used in this study was provided by The Arabidopsis Information Resource (TAIR) database and obtained from The Arabidopsis Biological Resource Center (ABRC) at Ohio State University through the TAIR website.

Mutant isolation

Mutants were grown under low intensity white light conditions at 12 μmol m^{-2} s^{-1} at 22°C for 7 days. Plants exhibiting visually discernable *shl* characteristics (short hypocotyls) were selected. Selected lines were further screened through multiple generations to confirm heritability, and in darkness to differentiate them from constitutively photomorphogenetic mutants.

Mutant characterization

The number of functional T-DNA insertions was determined by basta resistance ratios of F_2 backcrosses of T_2 plants to wild-type Col-0. Seeds were plated on MS/phytagar/2% sucrose media supplemented with glufosinate- ammonium (Basta) to a concentration of 7.2×10^{-4} %. The dominant/recessive status of the *shl* phenotypes were determined by the segregation of the phenotype in F_2 generations obtained from backcrosses to wild-type Col-0. The *shl* phenotypes were determined under low-white light conditions, and yellow light. Yellow light functionally simulates low-white light by filtering out the

photomorphogenically active spectra of light. Seedlings were grown for 7 days in the indicated light conditions.

TAIL PCR and plasmid rescue

DNA was extracted from candidates using the DNeasy Plant Mini kit (Qiagen, Valencia, CA USA). The *Arabidopsis* sequence flanking T-DNA insertion sites were isolated using Thermal Asymmetric Interlaced (TAIL) PCR as described (Liu *et al*. 1995) with the GSP primers LBRAA2, LBRA2, and LBRB2. Plasmid rescue was performed as described (Behringer *et al*., 1992) using *Spe*I and *Hind*III (New England Biolabs).

CHAPTER III

CHARACTERIZATION OF *SHL7*

INTRODUCTION

A previous screen of seed pools from 84,000 individual T_1 T-DNA lines in low light conditions yielded five new mutants with a *shl*, or *seedling hyper-responsive to light* phenotype. Based on several technical and biological criteria, including the strength of the phenotype and situation of the T-DNA insert, the *shl7* line in particular was awarded the highest priority for further study. The s*hl7* mutant has a consistent *shl* phenotype in low-white light. There is a single functional T-DNA insertion locus within the annotated gene, At4g04925. The *shl7* mutant is the focus of the work outlined in this section.

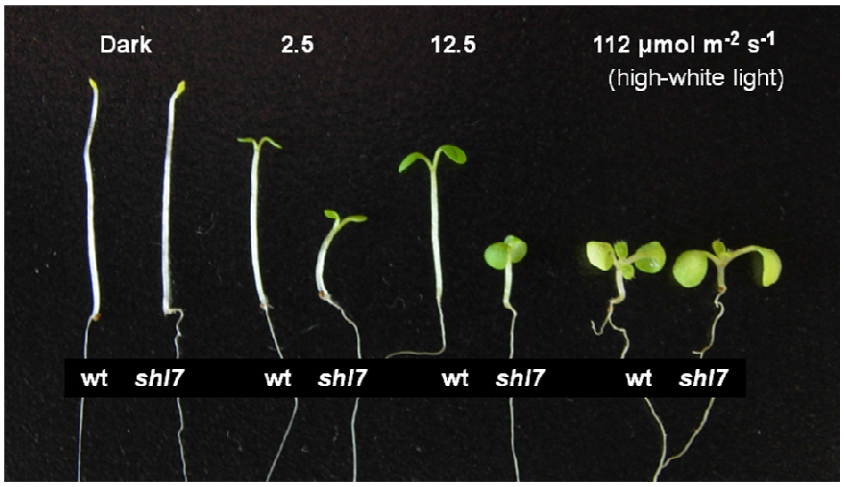
RESULTS

Identification of the shl7 mutational insert

To identify new members of the *seedling hyper-responsive to light* class of mutation, seed pools from 84,000 individual T_1 T-DNA lines were screened. An *Arabidopsis* light-responsive mutant seedling, identified during a low-fluence white-light screen, had a de-etiolated morphology with enhanced inhibition of hypocotyl elongation after 7 days of

growth. The phenotype was heritable as a single recessive mendelian allele through multiple generations and the mutant was subsequently named *shl7*. The *shl7* mutant displayed shorter hypocotyl lengths than ecotype Columbia when grown in low-white conditions (12 μmol m^{-2} s^{-1}), but in darkness showed negligible hypocotyl length deviations from wild-type Columbia (Figure 3.1). These findings were consistent with the *shl* phenotypic criteria (Pepper et al. 2001) distinguishing *shl7* from the *cop/det/fus* class. The T-DNA insertion contained a gene conferring resistance to the herbicide glufosinate, or Basta. Basta resistance ratios for *shl7* showed 54 alive to 18 dead plants with a χ^2 =0.0, suggesting a single locus of insertion for the functional T-DNA constructs. The mutant *shl7* gene segregated as a recessive allele during analysis of crosses to wild-type Columbia with a ratio of 37:14 wild-type long to mutant short hypocotyls, with a χ^2 =0.16 for an expected recessive allele, again suggesting the T-DNA insertion mutation results in a loss of function mutation (rather than an activation-tag induced overexpression).

(a)



(b)

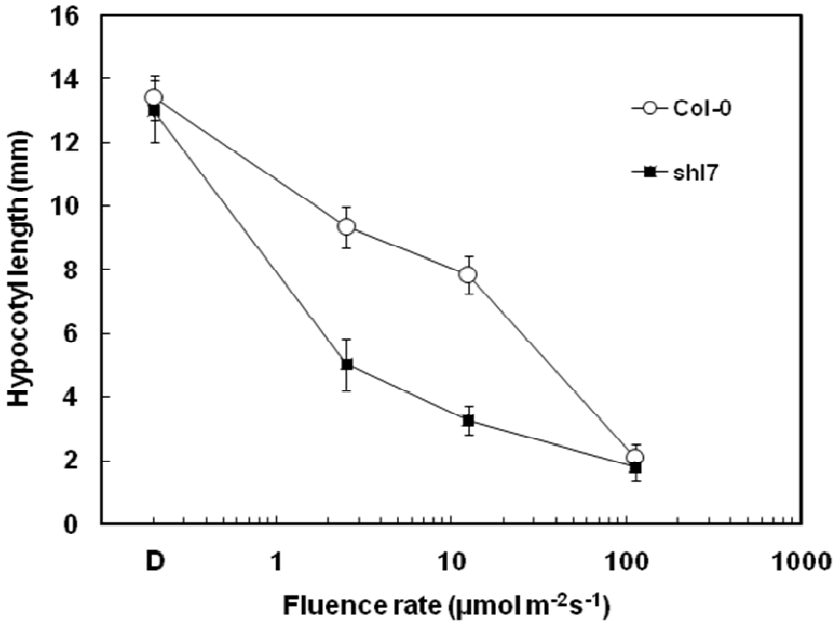
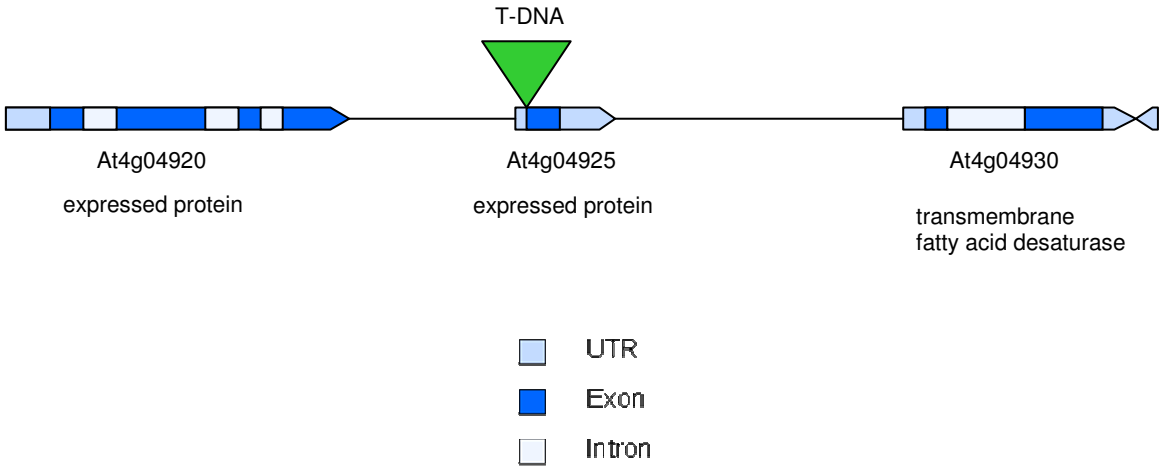


Figure 3.1 Morphologies of wild-type and *shl7*. Seedlings were grown for 7 days on Morishige and Skoog/phytagar/2% sucrose media.
(a) Morphologies in darkness, low-white light at fluences of 4 µmol $m^{-2}s^{-1}$ and 12 µmol $m^{-2}s^{-1}$, and in high-white light at a fluence of 100 µmol $m^{-2}s^{-1}$.
(b) Comparison of hypocotyl lengths of wild-type and *shl7* in darkness, low-white light at fluences of 4 µmol $m^{-2}s^{-1}$ and 12 µmol $m^{-2}s^{-1}$, and in high-white light at a fluence of 100 µmol $m^{-2}s^{-1}$. Error bars represent standard deviation.

Sequence analysis of flanking genomic DNA recovered from *shl7* plants by both plasmid rescue (Behringer, 1992) and TAIL PCR (Liu et al., 1995) identified a T-DNA insertion site within the 5′UTR of the annotated gene "expressed protein" At4g04925. To confirm that this T-DNA insertion was the cause of the *seedling hyper-responsive to light* phenotype of *shl7*, the mutant phenotype was complemented by reintroducing, through T-DNA transformation, the endogenous sequence derived from wild-type Columbia containing the annotated At4g04925 gene and ±1.87 kb of the regions flanking it. Reintroduction of this endogenous wild-type sequence into the *shl7* mutant resulted in the recovery of a wild-type phenotype in two separate lines (Figure 3.2). The successfully complemented *shl7* mutants displayed normal hypocotyl length when grown in low-white light conditions, and segregation ratios of the wild-type phenotype in T_2 generations were that of a dominant wild-type allele with 38 long wild-type hypocotyls to 13 short with χ^2 = .01 for an expected 3:1 ratio.

(a)



(b)

MRLGGFRRATWWLTADLFGSA**MA**NRDSRLIRVVGATVPVSLAIWFPLVRIDLD
WSREGCVVHRTTEGGYILHSSIC

(c)

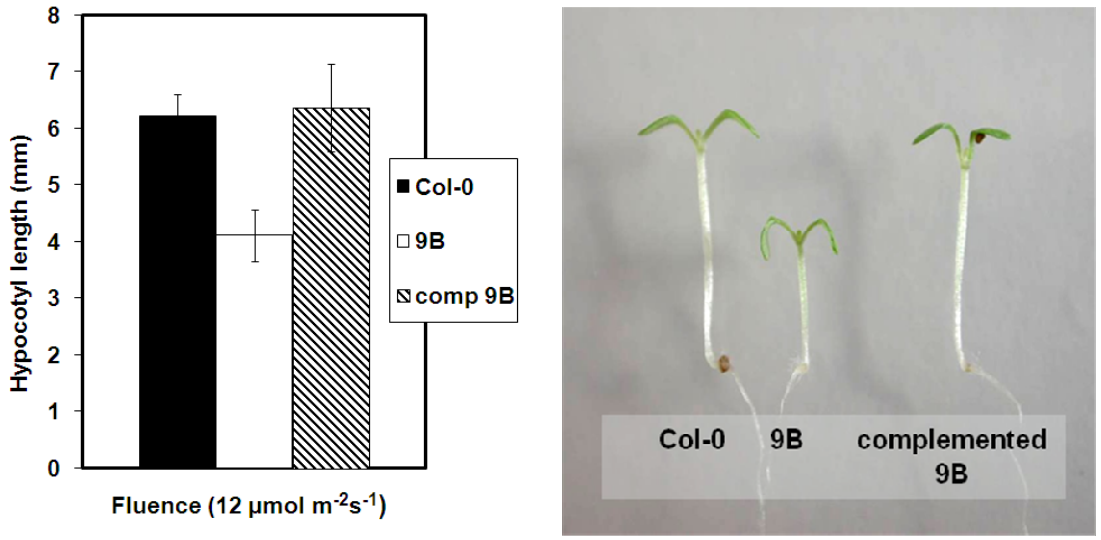
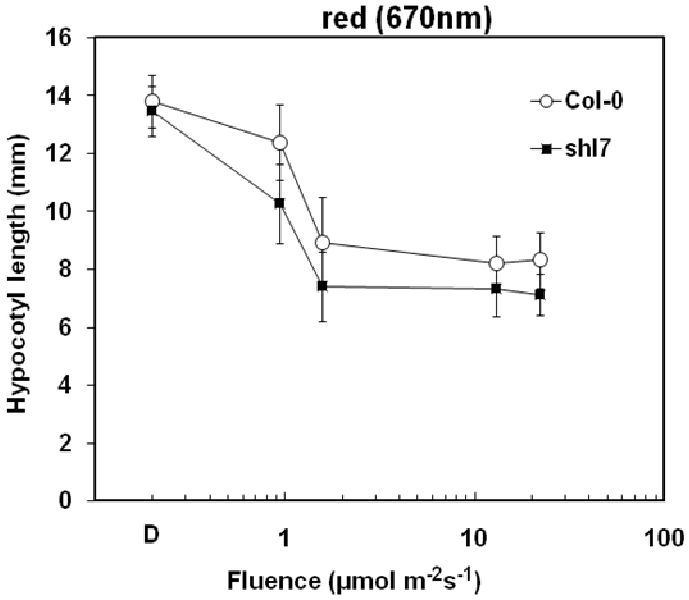


Figure 3.2 Wild-type *SHL7* gene structure and protein sequence. Complemented mutant *shl7* phenotype.
(a) *SHL7* gene structure, region, and T-DNA insertion locus.
(b) Amino acid sequence of SHL7 including predicted cleavage site of protein (in red).
(c) Recapitulation of wild-type phenotype in mutant by insertion of complementing wild-type DNA fragment.

Effects on photoperception

Seedling photomorphogenesis is primarily controlled by the photoreceptors that monitor the red/far-red spectra of light via phytochromes and the blue/UV-A spectrum via the cryptochromes. To determine if the *shl7* mutant phenotype was dependent on these particularly important wavelengths of light, and possibly their corresponding photoreceptors, *shl7* mutants were grown in narrow-spectrum red, far-red, and blue regions of the spectrum under varied fluences. Plants were also grown under varied fluences of white light. As with the wild-type, all of the light conditions tested resulted in inhibition of hypocotyl elongation. The *shl7* mutants grown under a range of fluence rates exhibited the strongest hyper-responsive phenotype in white light (Figure 3.1). Of the narrow-spectrum wavelengths, blue light elicited the strongest hyper-responsive phenotype, with significant effects on the inhibition of hypocotyl elongation. The far-red and red narrow-spectrum plants did display a hyper-responsive phenotype, but it was not statistically significant (Figure 3.3).

(a)



(b)

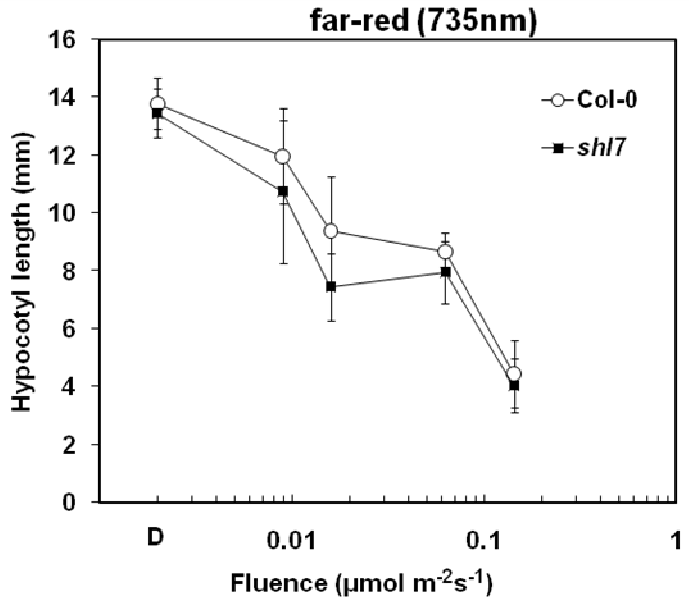


Figure 3.3 Morphologies of wild-type and *shl7*. Seedlings were grown for 7 days on Murishige and Skoog/phytagar/2% sucrose media, in narrow-spectrum light conditions. Error bars indicate standard deviations.
(a) red 670nm
(b) far-red 735nm
(c) blue 420nm

(c)

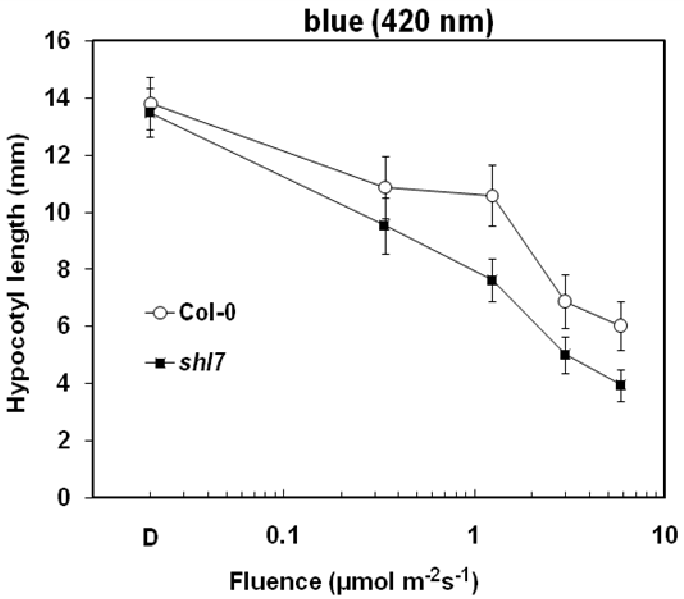


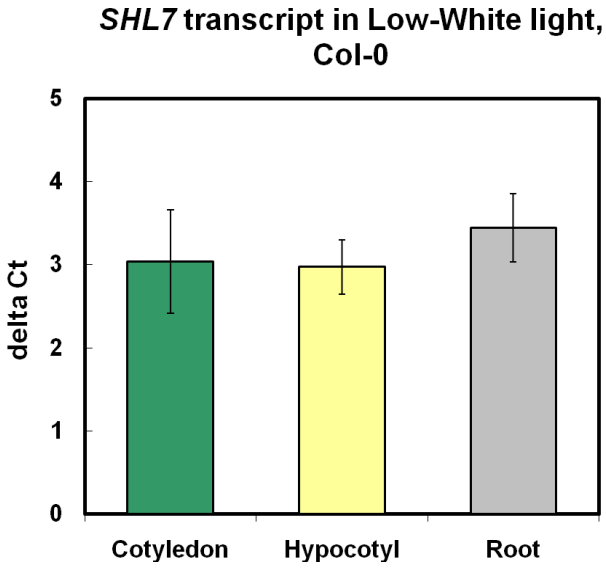
Figure 3.3 continued

Analysis of the SHL7 transcript in Col-0

The primary phenotype of the *shl7* mutant is observed in the seedling stage, and is visible as a difference in the length of the hypocotyl as compared to wild-type. Inhibition of hypocotyl elongation is a response to light perception. To gain insight concerning *SHL7's* influence on the morphology of the hypocotyl, organ-specific expression levels of the *SHL7* transcript were measured in wild-type seedlings grown in low-white light (12 μmol m^{-2} s^{-1}). RNA was isolated from cotyledons, hypocotyls, and roots and quantitative PCR was performed(). The *SHL7* transcript was found to be in relatively equal abundance within all three of the tissue types (Figure 3.4), suggesting that organ-specific transcriptional regulation may not be a key factor in the gene's function.

The photomorphogenic phenotype of *shl7* was most prominent in seedlings grown in low-white light conditions. To test for light-intensity dependent changes in transcript levels, RNA was extracted and pooled from ~200 wild-type Col-0 seedlings grown in dark, low-white (12 μmol m^{-2} s^{-1}), and high-light (100 μmol m^{-2} s^{-1}) conditions. The *SHL7* transcript was found to be in relatively equal abundance within the dark and low-white light conditions, but showed a three fold decrease in high-white light intensities, as compared to dark (Figure 3.4). Thus regulation of the *SHL7* transcript does appear to be influenced by light intensity.

(a)



(b)

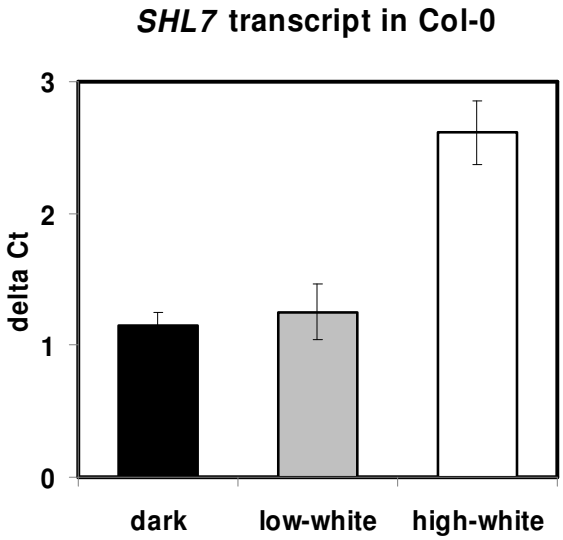


Figure 3.4 *SHL7* transcript in Col-0
Error bars represent standard deviations of four technical replicates
(a) Tissue specific *SHL7* transcript isolated from cotyledons, hypocotyls, and roots of Col-0
(b) *SHL7* transcript in Col-0 seedlings grown in darkness, low-white light at 12 µmol $m^{-2}s^{-1}$, and high-white light at a fluence rate of 100 µmol $m^{-2}s^{-1}$.

Analysis of the SHL7 transcript in shl7

The T_2 segregation of the *shl7* phenotype indicates that the phenotype of the *shl7* mutant is the result of a knockout mutation (T-DNA insertion) in the 5′ UTR of the At4g04925 gene. The restoration of the wild-type phenotype through the complementation experiments is expected to result in the restoration of the wild-type transcriptional profile. To confirm this, RNA was also extracted from seedlings from the *shl7* mutant and the complementation line discussed previously that was created via the re-insertion of the endogenous *SHL7* region into a mutant shl7. Seedlings were grown in dark, low-white (12 μmol m^{-2} s^{-1}), and high-white (100 μmol m^{-2} s^{-1}) conditions. RT-PCR was performed on all the samples including Col-0. The results of the transcript analysis indicated that the *shl7* T-DNA insertion mutant had a transcript being produced in the *SHL7* gene, albeit at somewhat lower levels than the wild-type Col-0. The complemented mutant had transcript levels higher than that of Col-0, as was expected (Figure 3.5).

The sequence immediately 5′ of the annotated *SHL7* gene (At4g04925) shows cDNAs and ESTs in the reverse orientation which truncate within 55 base pairs of the 5′ end of the *SHL7* gene and corresponding cDNAs and ESTs. The sequence of this 55bp gap indicates there is an A^{22} repeat followed by a T^{11} repeat. Available information regarding annotated cDNAs and ESTs show many of them terminating at these repeats, and none of them extending across the repeats. The A^n repeats could potentially act as binding sites for oligo dT primers commonly used in the cDNA synthesis of mRNAs. It is possible the transcript observed in the *shl7* mutant does not correspond to the endogenous *SHL7* gene transcript, but represents a transcript originating from an adjacent

transcript transcribed in the complementary direction, which also would have been picked up by the random hexamer primer used in the cDNA synthesis. The observed transcript may likewise be originating from the T-DNA insertion itself.

To determine the origin of the transcript observed in the *shl7* mutant, a primer set was designed flanking the T-DNA left border (LB) insertion site. RT-PCR was performed on the *shl7* mutant, the complementation line, and Col-0. The results indicated a transcript is indeed spanning the *SHL7* gene and the T-DNA insert (Figure 3.6). To determine whether this transcript is originating from the T-DNA insert or spanning the *SHL7* gene into the T-DNA insert, a gene-specific primer was used to reverse transcribe cDNA specific to any mRNA in the orientation of the *SHL7* gene as well as any transcript originating from the T-DNA. RT-PCR was performed and the results mirrored those of the original random hexamer-derived RT-PCR experiments (Figure 3.6), indicating the transcript seen in *shl7* mutants is originating from the T-DNA insertion.

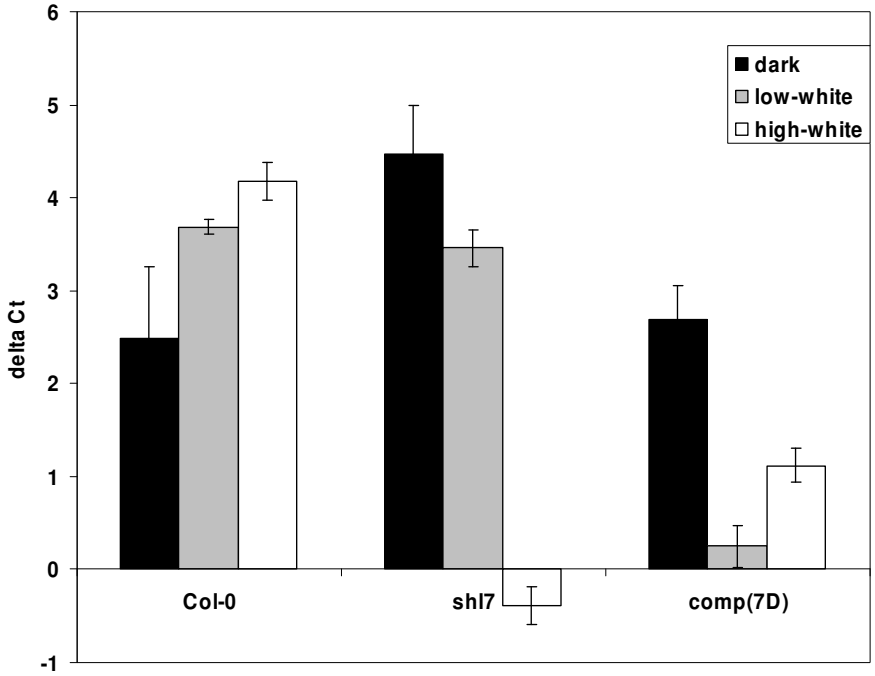
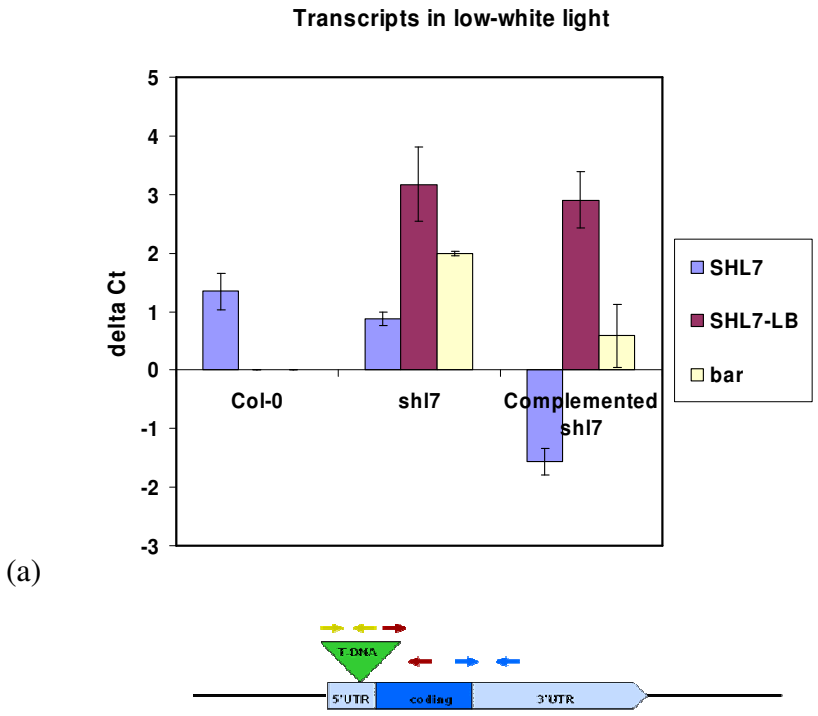
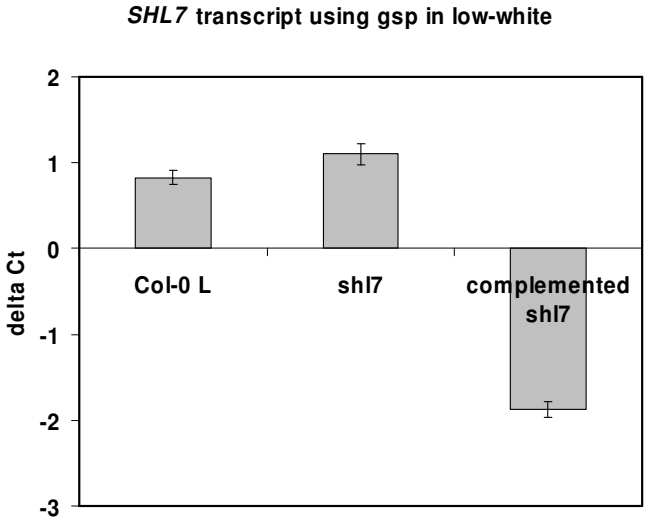


Figure 3.5 *SHL7* transcript in Col-0, *shl7* mutant, and the complemented *shl7* mutant, in dark, low-white, and high light conditions. Error bars are standard deviation of three technical replicates.



(a)



(b)

Figure 3.6 Transcripts in Col-0, *shl7* mutant, and the complemented *shl7* mutant. Error bars indicate the standard deviation of three technical replicates
(a) *SHL7* transcript, T-DNA LB to *SHL7* spanning transcript, and basta resistance gene (*bar*) transcript from T-DNA inserts. Transcript spanning the T-DNA LB to *SHL7* is present in T-DNA mutants.
(b) *SHL7* transcript results from cDNA reverse transcribed using a gene specific primer in the *SHL7* gene mirror hexamer derived results.

Subcellular localization using GFP fusion

To elucidate the mechanism of the *SHL7* gene's influence on hypocotyl morphology, the sub-cellular location of the SHL7 protein was observed. The TargetP localization prediction algorithm (Emanuelsson et al., 2000) predicted a mitochondrial localization of the protein product with a confidence of 0.96. To verify the mitochondria as the target destination of the SHL7 protein, a GFP tagging approach was used to visualize the protein's location in living cells. An SHL7/GFP protein fusion was constructed and transformed into wild-type Columbia (Col-0). The red fluorescent dye MitoTracker Red CMXRos, which is dependent upon membrane potential for accumulation in the mitochondrion of living cells, was selected as a marker for the comparison with the green GFP signal. The SHL7/GFP *Arabidopsis* seedlings were grown for 5 days in dark, low-white (12 μmol m^{-2} s^{-1}), and high-light (100 μmol m^{-2} s^{-1}) conditions and stained with the MitoTracker dye prior to viewing. Plants containing the SHL7/GFP protein were observed using confocal fluorescence microscopy. The resulting images indicated distinctive GFP signal accumulation that co-localized with the MitoTracker dye. The GFP signal was specific to the mitochondria and was not observed in any other organelle, nor did it appear to be present in visible quantities in the cytoplasm or nucleus (Figure 3.7). The SHL7/GFP protein appeared to be localized to the mitochondria in all of the samples from dark, low-white, and high-white light grown seedlings.

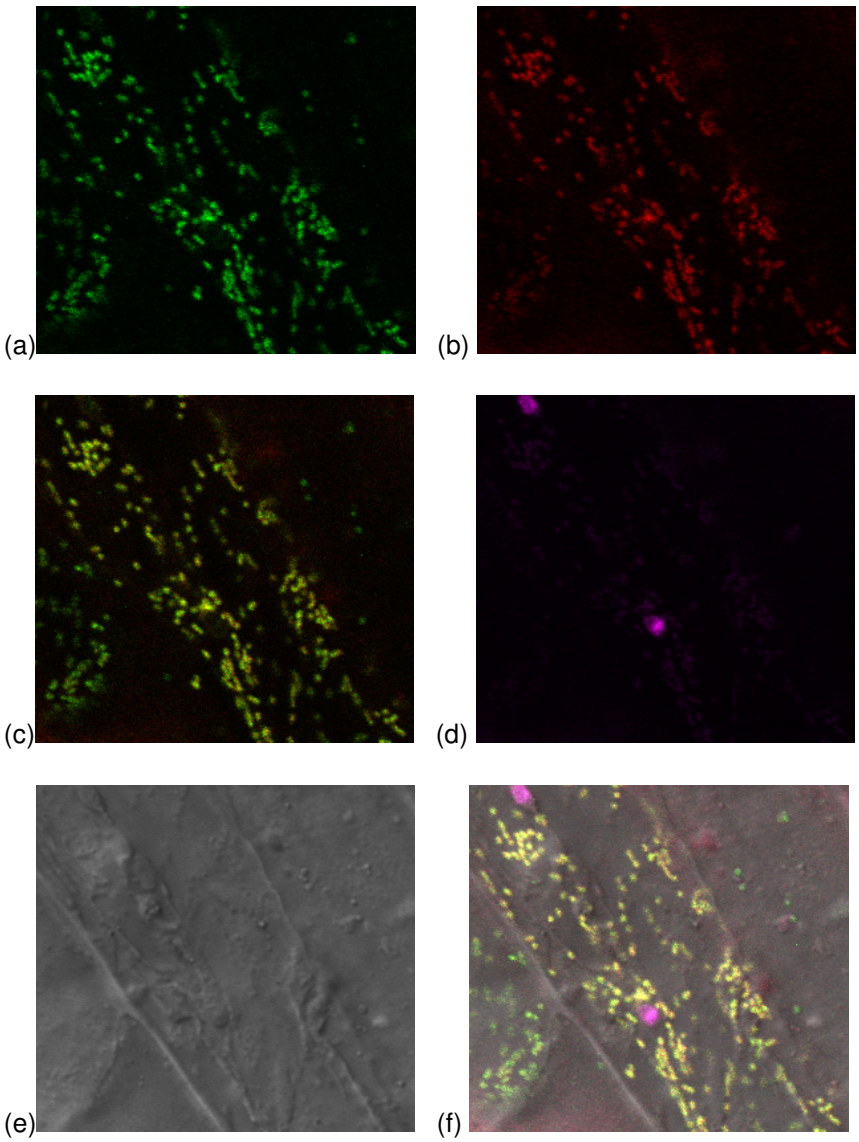


Figure 3.7 Confocal fluorescence microscopy of SHL7-GFP fusion in *Arabidopsis* hypocotyls grown in low-white light at 12 µmol $m^{-2}s^{-1}$.
(a) GFP
(b) MitoTracker
(c) GFP and MitoTracker channels overlaid
(d) Chloroplasts
(e) Nomarsky- differential interference contrast (DIC)
(f) All channels overlaid

DISCUSSION

shl7 is hyper-responsive to blue light

The *shl7* mutant showed light-dependent hyper-responsiveness of hypocotyl elongation in all light conditions tested. The *shl7* mutants grown under varied fluence rates of white light exhibited the strongest hyper-responsive phenotype. Of the narrow-spectrum wavelengths, blue light elicited the greatest hyper-responsive phenotype, with significant effects on the inhibition of hypocotyl elongation. This finding implicates the action of a blue light photoreceptor, such as a cryptochrome, on expression of the *shl7* phenotype. The far-red and red narrow-spectrum grown plants did exhibit a hyper-responsive phenotype, but to a much less significant degree. The fact that far-red and red light elicited a minor phenotypic response does not contradict the implication of a cryptochrome playing a predominant role in the actions of *SHL7*, due to known cross-talk between the phytochrome and cryprochrome signaling pathways. Responses were greatest in the low-white light conditions. This finding may be the result of additive or synergistic effects of the responses elicited by both the red and blue regions of the light spectrum.

The shl7 transcript is not tissue specific and is ubiquitiously expressed in seedlings

Real-Time PCR performed on cDNA from cotyledons, hypocotyls, and roots of seedlings

grown in low-white light identified the *SHL7* transcript as present in all three of the tissues examined. The transcript was found in relatively equal abundance in each of the tissues. The *SHL7* gene transcript does not appear to be tissue specific, indicating that the influence of *SHL7* on hypocotyl length in seedlings is not the result of the presence or absence of the *SHL7* transcript relative to other tissues. The entire *SHL7* gene transcript consists of a single unbroken coding sequence with no exons, so alternate splicing is unlikely to convolute these findings.

The transcription of the *SHL7* gene appears to be regulated by light. Analysis of seedlings grown in dark, low-white, and high-white light conditions revealed a decrease in *SHL7* mRNA quantities in the higher light intensities. The expression of the *SHL7* gene transcript in wild-type Columbia seedlings is decreased by a threefold margin in high-white light compared to dark. This finding reveals that transcriptional regulation of *SHL7* is occurring in a photo-dependent manner. The plants may be highly sensitive to the level of SHL7- a threefold increase in the protein could alter a sensitive stoichiometric interaction between SHL7 and another protein. An alternate hypothesis is that the SHL7 protein, rather than the quantities of it, is the mechanism of affecting a phenotype. The protein may be acting in a light-dependent manner, or acting upon a light-dependent target, such as a cryptochrome.

The presence of an SHL7 transcript in the *shl7* mutant appears to be the result of a transcript originating from the T-DNA insertion responsible for the *shl7* mutant. While there may be a transcript present, there is no indication that the transcript encodes a viable SHL7 protein. To address this issue, synthetic miRNA constructs designed to target *SHL7* mRNA for degradation have been made, and transformed into Col-0. This

will potentially confirm the phenotype of a definitive knock-out of *SHL7*.

Shl7 is localized to the mitochondria

Observation of the SHL7/GFP fusion product conclusively demonstrated the mitochondria as the target organelle of the SHL7 protein. There was no evidence of accumulation of the SHL7/GFP fusion product outside of the mitochondria, so its presence anywhere else, such as en route, was in quantities below the detection capabilities of the equipment. The SHL7/GFP protein fusion was found to be localized to the mitochondria in seedlings grown in dark, low-white, and high-white light conditions. This evidence suggests that the accumulation of the SHL7 is not light-dependent. It should be noted, however, that some light contamination likely occurred during the staining process, and as the seedlings were live plants, protein trafficking was free to continue during the actual microscopy process wherein each of the seedlings was exposed to uniform light conditions generated by the specific lasers used.

The functional role of the SHL7 protein in the mitochondria is unclear, but leaves open a wide range of possibilities. The mitochondria are best known for generating the majority of the ATP in a cell, but have also been shown to be an integral part of many cell-signaling cascades, and have involvement with a wide range of other processes including cellular differentiation and apoptosis (McBride *et al*., 2006). Mitochondria, through the action of bi-directional communication with the rest of the cell via GTPases, kinases, and phosphotases, can also be linked to cell cycle control and development (McBride *et al*., 2006). Recently, a putative mechanism of direct light perception in

mitochondria was reported (Wong-Riley *et al*., 2005), suggesting that red/near-infrared light can be absorbed by and modify the activity of cytochrome *c* oxidase in cultivated neurons. An unknown protein such as SHL7, situated in the mitochondria, has the potential to influence plant development through such a mechanism.

The presence of the SHL7 protein in the mitochondria is significant, regardless of whether its accumulation there is light dependent. *SHL7* is unique; no similar genes have been described which could provide insight into the function of the gene. The hyper-responsiveness of the *shl7* mutant to narrow-spectrum blue light is indicative of cryptochrome involvement. It is unlikely that SHL7 interacts directly with any of the major photoreceptors, as most photoreceptors reside in the nucleus, cytosol, or plasma membrane, with some photoreceptors communicating between these compartments upon exposure to light (Chen *et al*., 2004). It is possible that there may be interactions between SHL7 and some other protein or transcription factor lying within the downstream signaling pathway of one of these major photoreceptors.

There is only an intriguing possibility of direct interaction between SHL7 and a photoreceptor, which involves the most recent addition to the characterized cryptochrome family, *CRY3*. CRY3 has been localized to chloroplasts and mitochondria (Kleine *et al*., 2003), indicating that these organelles may directly perceive light. No evidence of *cry3*-mediated control of chloroplast or mitochondrial gene expression has yet been reported, and the overall function of this photoreceptor remains unknown. The mitochondrial localization of CRY3 places it in the proximity of SHL7, therefore direct interaction of the two proteins is plausible. To test this hypothesis, synthetic miRNA constructs designed to target *CRY3* mRNA for degradation have been made and been transformed

into Col-0 and *shl7*. If *SHL7* is involved in any direct or downstream interactions of *CRY3*, these miRNA lines have the potential to reveal that. A *shl7/cry3* double mutant can be screened in appropriate blue light conditions alongside *shl7* and *cry3* single mutants to ascertain any deviations between a single mutation and a double mutation. To test specifically for direct interactions, other avenues should be explored. There is plenty enough GFP/SHL7 protein produced in the *GFP/SHL7* lines to attempt using a GFP antibody to capture the GFP/SHL7 protein and see if CRY3 comes with it.

EXPERIMENTAL PROCEDURES

Plant lines and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used in all experiments. Plant lines with T-DNA insertion mutations were obtained from Dr. Rick Amasino and Dr. Scott Michaels (University of Wisconsin - Madison). Plants were grown in soil (Metromix 300) or on MS/phytagar/2% sucrose media in 25 mm x 100 mm polystyrene Petri dishes. Seeds were surface sterilized (Chory *et al*., 1989), resuspended in 0.1% phytagar, and cold-treated for 48 hours prior to plating. Seeds were evenly dispersed onto solid agar media in a 7mm grid pattern, and then subjected to 4 hours of white light (100 μmol m^{-2} s^{-1}) prior to placement into specified light conditions.

For white and low-white light conditions, plants were placed in a temperature-

and humidity-controlled growth room under GE Cool White and Sylvania Gro Lux lights at 22±1°C. Neutral density filters were used to adjust fluence rates for low-white light. For red, far-red, and blue light conditions, plants were placed in Percival growth chambers at 22±1°C. Red light was delivered by a Quantum Devices LED array model SL515-670 [670-nm maximum]. Far-red light was delivered by a Quantum Devices LED array model SL515-735 [735-nm maximum]. Blue light was delivered with Coralife Actinic Blue 7100K lights and Kopp 5-57 glass filters [420-nm maximum] with neutral density filters (Lee Filter #209) to adjust fluence rates. Fluence rates of white, red, and blue light were measured with a quantum photometer (model LI-189, LI-COR, Lincoln, NE). Fluence rates of far-red enriched light were measured using a radiometer (model IL400, International Light, Newburyport, MA) with a far-red probe (model SEL033, International Light).

Complementation lines

Complementation was performed using a 1868bp fragment of DNA containing the *SHL7* expressed sequence as well as 1015bp of upstream sequence (that presumably included much, if not all, critical promoter sequences). The endogenous sequence from the putative *SHL7* gene location was PCR-amplified from Col-0 DNA with the primers 9B-F4 and 9B-R using the high fidelity polymerase Phusion (New England Biolabs), and cloned into the PCR blunt II Topo vector (Invitrogen). The complementation construct was generated by ligation of the *Eco*RI SHL7 fragment into pCAMBIA 3300 cut with *Eco*RI (New England Biolabs). Mutant *shl7* lines were transformed with the construct

via the floral dip method as described (Bechtold *et al*., 1993) using *Agrobacterium* strain GV3101. T_0 seeds were individually potted in soil and DNA was harvested from ~2.5 mg of leaf tissue from resulting plants using a Sucrose Prep nucleic acid extraction protocol as described (Berendzen *et al*., 2005). Transformants were screened using the primer set M13-F and 9B25Rt-F, which was specific to the pCAMBIA T-DNA and *SHL7* sequence.

RT-PCR analysis

RNA was isolated from seedlings using the RNeasy Plant Mini kit (Qiagen, Valencia, CA USA), and RNA concentrations were determined using a Beckman DU60 Spectrophotometer. An off-column DNaseI treatment was done using a DNA*free*™ kit (Ambion, Austin, Texas) to remove any DNA. RNA was isolated from 5-day-old seedlings grown in dark, low-white, and high-white light. Total cDNA was reverse transcribed from 700ng of RNA using a SuperScriptIII First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) with a random hexamer primer. Quantitative PCR was performed on dilute cDNA using Power SYBR master mix (Applied Biosystems) and an ABI 7900HT real-time (RT) PCR machine. The *SHL7* gene was amplified using primers 9B25RT-F and 9B25RT-R. The *ELONGATION FACTOR1 (EF1)* gene was used as an internal control. Tissue-specific RT-PCR analysis was done as previously stated, except that RNA was isolated from dissected cotyledons, hypocotyls, and roots from approximately 90 seedlings.

Table 3.1 Primers used in TAIL PCR, RT-PCR, complementation, and GFP protein fusion experiments.

Name	Sequence (5′ > 3′)
LBRAA2	CCATTTGGACGTGAATGTAG
LBRA2	ATTGCTTTCGCCTATAAATACGAC
LBRB2	AATAACGCTGCGGACATCTAC
9B-F4	AACAGGTCTTGGTGTATAGCG
9B-R	GCCTAAGCCTCTATTCTTGAG
M13-F	CAGGGTTTTCCCAGTCACGAC
9B25RT-F	GGTTACATCTTGCATTCATCG
9B25RT-R	GGAATAAGTCAAAACTCCGAC
EF-F	TCGAATCCTCAAAACTCTACCGCA
EF-R	GGAGAAGAAGAAACGAAGCTATTA
BarRT-F	GATGACAGCGACCACGCTCTTG
BarRT-R	ACTGGGCTCCACGCTCTACAC
9BLbRT-F	GACCATCATACTCATTGCTGATC
9BLbRT-R	GCAAGTATCGGATAAACCTATCC
9BbamG-F	AAAGGATCCAAAGTGTCTTCCAATCAACCCCG
9BbamSG-R	CAAGGATCCGCAAATCGATGAATGCAAGATG

Mutant characterization

The single T-DNA insertion locus was determined by basta resistance ratios of backcrosses to wild-type Col-0. The recessive status of the *shl7* phenotype was determined by segregation of the phenotype in F_2 generations obtained from backcrosses to wild-type Col-0. Phenotypes of wild-type and *shl7* mutants were determined under low-light conditions and varied fluence rates of red, far-red, and blue light. Seedlings were grown for 7 days in the indicated light conditions. The insides of 25 mm x 150 mm polystyrene petri dishes were coated with a 0.5 mm layer of agarose/synergel dissolved in water; this provided a substrate to which seedlings would adhere, and prevented dessication. A 1mm scale grid was affixed to the bottom of these plates which was clearly visible through the agarose/synergel. Seedlings were then removed from the MS/phytagar/2% sucrose petri dishes and placed flat onto the agarose/synergel layer in the new petri dishes. The seedlings' hypocotyls were straightened and aligned along the 1mm grid. A digital photograph was taken of the seedlings on the grid with a Nikon Coolpix 900 camera, from a distance of 0.5 meters. The image was analyzed and the measurements were assigned and recorded using the Microsoft Paint program (Version5.1). Seedlings with obvious developmental abnormalities were not measured.

Subcellular localization of SHL/GFP fusion

A GFP/SHL7 translational fusion was constructed using the synthetic GFP construct (Chiu *et al*., 1996; Hasslehof *et al*., 1998) and the binary vector pCBK05 (K.Riha and

D.E.Shippen, unpublished data). The binary vector pCBK05 contains a 35S Cauliflower Mozaic Virus (CaMV) promoter sequence (Benfey and Chua, 1990) that is commonly used to generate large quantities of transcript which are significantly larger than the average endogenous plant promoter. A *Bam*HI *Sac*I GFP fragment was ligated into pCBK05 cut with *Bam*HI and *Sac*I. A *Bam*HI *SHL7* gene sequence fragment was PCR amplified from Col-0 using the primers 9BbamG-F and 9BbamSG-R with the high fidelity thermostable polymerase Phusion (New England Biolabs) and ligated in-frame with the GFP sequence of pCBK05 cut with *Bam*HI. The completed GFP/SHL7 fusion vector was transformed into *Agrobacterium* strain GV3101 for use in plant transformation. Col-0 plants were then transformed via *Agrobacterium* infiltration (Bechtold *et al*., 1993). Successful transformants were selected using the herbicide glufosinate (Basta) resistance. GFP/SHL7 6-day-old seedlings grown in dark, low-white, and high-light conditions were stained with MitoTracker Red CMXRos (Invitrogen) for 10 minutes in water at a 10 nM concentration, and rinsed in water. *GFP/SHL7* fusion transformants were viewed using the Olympus FV100 confocal system at the Texas A&M Microscopy Center. Plants were viewed using DIC (Nomarsky) illumination, and with filter sets for GFP and Cy3. Image analysis was done using the Olympus Imaging software FV10-ASW 1.6 Viewer.

CHAPTER IV

CONCLUSIONS

SCREENING

Mutant screen

Mutant screens designed to identify mutants defective in aspects of photomorphogenesis are a proven means of gathering new data on how light works as an environmental factor to govern plant morphology. Screens done in various light conditions can identify photoreceptors as well as downstream signaling components. A previous screen of EMS-generated mutants in low-white light conditions identified six mutants with hyper-responsive to light phenotypes. In this study, five more hyper-responsive to light mutants were identified from a similar low-white light screen. The locations of T-DNA inserts were deduced by inspecting the plant sequences flanking the inserts, which was recovered by TAIL PCR and/or plasmid rescue. The insertion sites of the T-DNA were not redundant with any previously characterized light regulated genes. This was not unexpected, as light influences the expression of a significant portion of the genes in the Arabidopsis genome. Low-white light mutant screens remain a useful avenue for the identification of additional light regulated genes.

This particular T-DNA mutant screen yielded multiple mutants with heritable phenotypes, which were not selected to for additional study and characterization. Any future eperiments would begin with these already screened lines, and would begin with

locating the T-DNA insertion sites of these other lines. Any unique mutations would be further characterized, as appropriate.

SHL7

shl7

The *shl7* mutant phenotype segregated like a recessive allele in F_2 generations of mutants backcrossed to wild-type Col-0. This is consistent with what would be expected of a loss- of- function or "knock-out" mutation. The T-DNA insertion in the *shl7* mutant contains a Basta resistance gene adjacent to the T-DNA left border. It appears that a transcript is transcribed from within the T-DNA borders across the *shl7* gene region. This transcript is detectable through quantitative PCR. It cannot be assumed that the transcript is successfully translating the *SHL7* protein. This transcript does, however make it difficult to use quantitative PCR to confirm that the endogenous *SHL7* transcript is knocked out.

To address this issue, a miRNA construct designed to knock out *SHL7* has been constructed and transformed into Col-0 and *shl7*. Future and ongoing work entails the analysis of these miRNA generated knockout lines to see if they knock out *SHL7* and recapitulate the current *shl7* phenotype.

shl7 and blue light

The SHL7 protein was localized to the mitochondria. It is assumed that this is where it is functioning. It may be possible that SHL7 is interacting with CRY3, which has also been observed to localize in the mitochondria, indicating that these organelles may directly perceive light. No evidence of *cry3*-mediated control of chloroplast or mitochondrial gene expression has yet been reported, and the overall function of this photoreceptor remains mysterious. The mitochondrial localization of CRY3 places it in the proximity of SHL7, therefore direct interaction of the two proteins is plausible.

In an effort to elucidate what effect, if any, CRY3 may have on the *shl7* mutant, miRNA constructs have been designed and transformed into Col-0 and the *shl7* mutant. Future and ongoing experiments include observing these miRNA lines in low-white and narrow spectrum blue light conditions. If SHL7 is interacting directly with CRY3, or if *SHL7* is involved in downstream signaling pathways from *CRY3*, there should be a loss of the *shl7* phenotype in a miRNA induced *cry3* background. The effects of a *CRY3* knockout by itself have not yet been determined, so even if there are no interactions between *SHL7* and *CRY3* the results will be informative.

REFERENCES

Ahmad, M., and Cashmore, A. (1993). HY4 gene of *Arabidopsis thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature.* **366,** 162-166.

Bechtold, N., Ellis, J. & Pelletier, G. (1993). In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C. R. Acad. Sci.* **316,** 1194–1199.

Benfey, P.N., and Chua, N.H. (1990). The cauliflower mosaic virus 35S promoter: Combinatorial regulation of transcription in plants. *Science*. **250,** 959-966.

Berendzen K., Searle I., Ravenscroft D., Koncz C., Batschauer A., Coupland G., Somssich I.E. and Ulker B. (2005). A rapid and versatile combined DNA/RNA extraction protocol and its application to the analysis of a novel DNA marker set polymorphic between Arabidopsis thaliana ecotypes Col-0 and Landsberg erecta. *Plant Methods*. Aug 23;1(1):4. ?

Behringer, F.J., and Medford, J.I. (1992). A plasmid rescue technique for the recovery of plant DNA disrupted by T-DNA insertion, *Plant Mol Biol.* **10**, 190-198.

Cashmore, A., Jarillo, J., Wu, Y. (1999). Cryptochromes: blue light receptors for plants and animals, *Science*. **284**, 760-765.

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet*. **38**, 87-117.

Chiu, W., Niwa, Y., Zeng, W., Hirano, T., Kobayashi, H. and Sheen, J. (1996). Engineered GFP as a vital reporter in plants. *Curr Biol.* **6**, (3), 325-330.

Chory, J., Peto, C., Feinbaum, R., Pratt, L., and Ausubel, F. (1989). *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell* **58,** 991-999.

Darwin,C. Darwin, F. (1880). The power of movement in plants. BOOK

Dieterle, M., Buche, C., Schafer, E., and Kretsch, T. (2003). Characterization of a novel non- constitutive photomorphogenic *cop1* allele. *Plant Physiology*. **133**, 1557-1564.

Eastmond, P. (2006). SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating *Arabidopsis* seeds. *The Plant Cell*. **18**, 665-675.

Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology*. **4**, 1005-1016.

Fankhauser, C., and Chory, J. (1997). Light control of plant development. *Annu. Rev. Cell Dev. Biol.* **13,** 203-229.

Genoud, T., Millar, A., Nishiawa, N., Kay, S., Schafer, E., Nagatani, A., Chua N. (1998). An Arabidopsis mutant hypersensitive to red and far-red light signals. *Plant Cell* **10,** 889-904

Haseloff, J., Siemering, K.R., Prasher, D.C. and Hodge, S. (1998). Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly. *Proc Natl Acad Sci U S A.* **94**, (6), 2122-2127.

Holm M, Ma LG, Qu LJ, and DengXW. (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of lightdependent gene expression in Arabidopsis. *Genes Dev.* **16**, 1247–59.

Kang, X., Chong, J., Ni, M. (2005). HYPERSENSITIVE TO RED AND BLUE 1, a ZZ-Type Zinc Finger Protein, Regulates Phytochrome B-Mediated Red and Cryptochrome-Mediated Blue Light Responses. *Plant Cell* **17**, 822-835.

Kang, X., and Ni, M. (2006). Arabidopsis SHORT HYPOCOTYL UNDER BLUE1 Crontains SPX and EXS Domains and Acts in Cryptochrome Signaling. *Plant Cell* **18**, 921-934.

Kim, S., Choi, H., Ryu, H., Park, J., Kim, M., Kim, S. (2004). ARIA, an Arabidopsis arm repeat protein interacting with a transcriptional regulator of abscisic acid-responsive gene expression, is a novel abscisic acid signaling component. *Plant Physiology*, **136**, 3639-3648

Kleine, T., Lockhart, P. and Batschauer, A. (2003). An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. *Plant J.* **35,** (1), 93-103.

Koornneef, M., Reuling, G., Karssen, C. (1984). The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. *Physiologia Plantarum***. 61**, 377-383.

Lin C. (2002). Blue light receptors and signal transduction. *Plant Cell* **14**, S207– S225.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J. (1998). Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. *PNAS.* **95**, 2686–2690.

Liscum E., Hodgson, D., and Campbell, T. (2003). Blue light signaling through the cryptochromes and phototropins. So that's what the blues is all about. *Plant Physiol*. **133,** 1429-1436.

Liu, Y.G., Mitsukawa, N., Oosumi, T., and Whittier, R.F. (1995). Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J*. **8,** 457-463.

Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H. and Xing, W.D. (2001) Light control of Arabidopsis development entrails coordinated regulation of genome expression and cellular pathways. *Plant Cell* **13**, 2589–2607

McBride, H.M., Neuspiel M. and Wasiak S. (2006). Mitochondria: more than just a powerhouse *Curr. Biol*. **16**, (14), 551-560.

Nam, K., and Li, J. (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell*. **110**, 203-212.

Neff, M., Fankhouser, C., Chory, J. (2000) Light: an indicator of time and place. Genes Dev. **14**, 257-271.

Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Yoshida, S., Yuan, H., Feldmann, K.A., and Tax, F.E. (1999). Brassinosteroid-insensitive dwarf mutants of *Arabidopsis* accumulate brassinosteroids. *Plant Physiol*. **121**, 743–752.

Osterlund, M.T., Hardtke, C.S., Wei, N., and Deng, X.W. (2000). Targeted destabilization of HY5 during light-regulated development of *Arabidopsis. Nature* **405,** 462-466.

Parks, B.M., and Quail, P.H. (1991). Phytochrome-deficient hy1 and hy2 long hypocotyl mutants of *Arabidopsis* are defective in phytochrome chromophore biosynthesis. *Plant Cell*. **3**, 1177-1186.

Pepper, A., Seong-Kim, M., Hebst, S.M., Ivey, K.N., Kwak, S.J., and Broyles, D.E. (2001). Shl, a new set of *Arabidopsis* mutant with exaggerated developmental responses to available red, far-red, and blue light. *Plant Physiol*. **127,** 295-304.

Quail, P.H. (2002). Phytochrome photosensory signaling networks. *Nature* **31,** 85-93.

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, et al. (2001). Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. *Proc. Natl. Acad. Sci. USA* **98**, 6969–6974.

Somers, D., Sharrock, R., Tepperman, J., Quail, P. (1991) the hy3 long hypocotyl mutant of Arabidopsis is deficient in phytochrome B. *Plant Cell*. **3**, 1263-1274.

Steindler, C., Matteuci, A., Sess, G., Weimar, T., Ohgishi, M., Aoyama, T., Morelli, G., and Ruberti, I. (1999). Shade avoidance responses are mediated by the ATHB-2 HD-Zip protein, a negative regulator of gene expression. *Dev*. **126,** 4235-4245.

Sullivan, J.A., and Deng, X.W. (2003). From seed to seed: the role of photoreceptors in *Arabidopsis* development. *Dev. Biol*. **260,** 289-297.

Wang H, and Deng XW. (2002). Arabidopsis FHY3 defines a key phytochrome A signaling component directly interacting with its homologous partner FAR1. *EMBO J.* **21**, 1339–1349.

Wang, X., and Chory, J. (2006) Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. *Science*. **313**, (5790), 1118-1122.

Wang, Z.Y., Seto, H., Fujioka, S., Yoshida, S., and Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* **410**, 380–383.

Weigel, D., Ahn, J.H., Blazques, M.A., Borevitz, J.O., Christense, S.K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil, E.J., Neff, M.M., Nguyen, J.T., Sato, S., Wang, Z.Y., Xia, Y., Dixon, R.A., Harrison, M.J., Lamb, C.J., Yanofsky, and M.F., and Chory, J. (2000). Activation tagging in *Arabidopsis*. *Plant Physiol*. **122,** 1003-1013.

Wong-Riley, M.T.T., Liang, H.L., Eells, J.T., Chance, B., Henry, M.M., Buchmann, E., Kane, M. and Whelan, H.T. (2005). Photobiomodulation directly benefits primary neurons functionally inactivated by toxins. *J. Biol. Chem.* **280**, (6), 4761-4771.

Zhao, Q., Leung, S. Corbett, A., and Meier, I. (2006). Identification and characterization of the *Arabidopsis* orthologs of nuclear transport factor 2, the nuclear import factor of ran. *Plant Physiol*. **140,** 869-878.

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